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MTC
QR 183.36

From: Rawlings, Stephen
Sent: Monday, November 03, 2003 3:32 PM
To: STIC-ILL
Subject: ill request

Art Unit / Location:	1642/CM1,8E17
Mail box / Location:	Rawlings - AU1642 / CM1, 8E12
Telephone Number:	305-3008
Application Number:	09589870

Please provide a copy of the following references:

1. Gallizia A, et al. Protein Expr Purif. 1998 Nov;14(2):192-6.
2. Dubel S, et al. J Immunol Methods. 1995 Jan 27;178(2):201-9.

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Stephen L. Rawlings, Ph.D.
Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
Mail Box - Room 8E12
(703) 305-308

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QH506.M65

From: Rawlings, Stephen
Sent: Monday, November 03, 2003 11:55 AM
To: STIC-ILL
Subject: ill request

Art Unit / Location:	1642/CM1,8E17
Mail box / Location:	Rawlings - AU1642 / CM1, 8E12
Telephone Number:	305-3008
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3. Jobling MG, et al. Plasmid. 1997;38(3):158-73.
4. Shibui T, et al. Appl Microbiol Biotechnol. 1993 Mar;38(6):770-5.
5. Shibui T, et al. Appl Microbiol Biotechnol. 1992 Jun;37(3):352-7.
6. Skerra, et al. Protein Eng. 1991 Dec;4(8):971-9.
7. Gray et al. J Cell Sci Suppl. 1989;11:45-57.
8. Tudyka T, et al. Protein Sci. 1997 Oct;6(10):2180-7.
9. Blight MA et al. Trends Biotechnol. 1994 Nov;12(11):450-5.

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Stephen L. Rawlings, Ph.D.
Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
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(703) 305-308

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QH506,E5

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Stephen L. Rawlings, Ph.D.
Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
Mail Box - Room 8E12
(703) 305-308

QR53.E9
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Stephen L. Rawlings, Ph.D.
Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
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QR53.09

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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
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(703) 305-308

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Suppl. NO 11/3

From: Rawlings, Stephen
Sent: Monday, November 03, 2003 11:55 AM
To: STIC-ILL
Subject: ill request

4/0536

Art Unit / Location:	1642/CM1,8E17
Mail box / Location:	Rawlings - AU1642 / CM1, 8E12
Telephone Number:	305-3008
Application Number:	09589870

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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
Mail Box - Room 8E12
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QP55 1.P697

From: Rawlings, Stephen
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Application Number:	09589870

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3. Jobling MG, et al. Plasmid. 1997;38(3):158-73.
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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
Mail Box - Room 8E12
(703) 305-308

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QP601.C73

From: Rawlings, Stephen
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Subject: ill request

Art Unit / Location:	1642/CM1,8E17
Mail box / Location:	Rawlings - AU1642 / CM1, 8E12
Telephone Number:	305-3008
Application Number:	09589870

Please provide a copy of the following references:

1. Kipriyanov SM, et al. Protein Eng. 1996 Feb;9(2):203-11.
2. Pearce LA, et al. Biochem Mol Biol Int. 1997 Sep;42(6):1179-88.
3. Kipriyanov SM, et al. Hum Antibodies Hybridomas. 1995;6(3):93-101.
4. Yao Z,et al. Nucl Med Biol. 1998 Aug;25(6):557-60.
5. Gandecha AR, et al. Gene. 1992 Dec 15;122(2):361-5.
6. Sano et al. J Chromatogr B Biomed Sci Appl. 1998 Sep 11;715(1):85-91.
7. Gallizia A, et al. Protein Expr Purif. 1998 Nov;14(2):192-6.
8. Sano T, et al. Biochem Biophys Res Commun. 1991 Apr 30;176(2):571-7.
9. Sano T, et al. Methods Enzymol. 2000;326:305-11.
10. Dubel et al. J Immunol Methods. 1995 Jan 27;178(2):201-9.

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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
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QR183, J6

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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
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(703) 305-308

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R895.A1 I56
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Patent Examiner, Art Unit 1642
Crystal Mall 1, Room 8E17
Mail Box - Room 8E12
(703) 305-308

L4: Entry 81 of 120 File: USPT Nov 17, 1998

DOCUMENT-IDENTIFIER: US 5837814 A
TITLE: Cellulose binding domain proteins

Application Filing Year (1):
1995

Brief Summary Text (45):
In a specific embodiment of the present invention, the method is designed for the detection of a protein or peptide; thus, the second protein of the CBD fusion product may be an antibody against the protein or peptide. The substance of interest may also comprise a biotinylated probe bound to a protein, peptide, hormone, nucleic acid or other probe-targetable molecule. In this case, the preferred second protein is streptavidin. Where the label includes an enzyme, the method further comprises adding a sufficient amount of a substrate for the enzyme, which substrate is converted by the enzyme to a detectable compound.

Detailed Description Text (2):
The present invention is directed to the identification of cellulose binding domain (CBD) protein that is capable of binding cellulose with high affinity and in a reversible manner. The CBD of the present invention may be used, for example, in the bio-immobilization of biologically active molecules to cellulose. The CBD of the present invention may be fused to a second protein to form a CBD fusion protein. The presence of a CBD protein in a CBD fusion protein allows for easy and selective purification of the CBD fusion protein by incubation with cellulose. Examples of second proteins include: Protein A, protein G, streptavidin, avidin, Taq polymerase and other polymerases, alkaline phosphatase, RNase, DNase, various restriction enzymes, peroxidates, glucanases such as endo-1,4-beta glucanase, endo-1,3-beta-glucanase, chitinases, and others, beta and alfa glucosidases, beta and alfa glucoronidases, amylase, transferases such as glucosyl-transferases, phospho-transferases, chloramphenicol-acetyl-transferase, beta-lactamase and other antibiotic modifying and degrading enzymes, luciferase, esterases, lipases, proteases, bacteriocines, antibiotics, enzyme inhibitors, different growth factors, hormones, receptors, membranal proteins, nuclear proteins, transcriptional and translational factors and nucleic acid modifying enzymes. Specifically, the CBD protein may be fused to an antibody or an antigenic determinant to form a CBD fusion product that is useful in diagnostic kits and in immunoassays.

Detailed Description Text (63):
The overexpression vector (pET-CBD) enables us to overproduce the 17 kDa CBD in E. coli strain BL21(DE3). CBD was accumulated to at least 70 mg/liter in inclusion bodies. However, additional quantity of about 20 mg/liter of water-soluble CBD could be recovered from the water-soluble sonic extract of the E. coli. The cleared extract was mixed with Sigmacell 20(20 micron average particle size cellulose); then the CBD-cellulose complex was washed by 1M NaCl solution as well as distilled water to remove non-specific proteins, and then pure CBD was eluted by 6M guanidine-HCl. CBD was fully renatured by slow dialysis at room temperature and regained its ability to bind to cellulose (FIG. 10. lane 2).

Detailed Description Text (65):
Plasmid DNA containing the insert was used to transform E. coli BL21 (DE3). Plasmid-containing cultures were grown at 37.degree. C. in NZCYM (Sambrook, et al. (1989) in Molecular Cloning (Nolan, C. ed.), Cold Spring Harbor Laboratory Press, N.Y. medium containing ampicillin (100 .mu.g/ml) with shaking to Klett reading 160 (green

filter). At this point, IPTG was added to a final concentration 1 mM. After 4 h, the cells were harvested by centrifugation, resuspended in 50 mM phosphate/12 mM citrate pH 7 (PC) buffer containing RNAse A at 10 .mu.g/ml and DNAse I at 1 .mu.g/ml, and lysed by sonication on ice with a Biosonic II sonicator at maximum power for 45 s followed by a 15 s cooling period, repeated a total of 4 times. The insoluble fraction of a 1 l cell culture was collected by centrifugation (30 min at 12,000 g, 4.degree. C.) and resuspended in 20 ml of 6M guanidine HCl. This was kept on ice for 30 min with occasional vortexing to disperse the pellet. Insoluble debris was removed by centrifugation (30 min at 12,000 g, 4.degree. C.). The soluble guanidine HCl extract was gradually diluted to 400 ml total volume with TEDG renaturation buffer over a two h period at 4.degree. C. Ammonium sulfate was added to 80% saturation. After four h at 4.degree. C., precipitated proteins were collected by centrifugation (30 min at 12,000 g, 4.degree. C.), resuspended in 40 ml PC buffer, and dialyzed against PC buffer.

Detailed Description Text (78):
In order to selectively produce the putative CBD region of CbpA (residues 28-189), oligonucleotide primers were designed complementary to bases 67 to 86 and 558 to 579 of cbpA (FIG. 1A-1B). As shown in FIG. 2, these primers were designed with mismatches to create an NcoI site and an ATG start codon on one end of the PCR product and a TAG stop codon followed by a BamHI site at the other end. This gene fragment was then cloned into the T7 RNA polymerase expression plasmid pET-8c, resulting in plasmid pET-CBD. See, Studier, F., and B.A. Moffatt (1986) J. Mol. Biol. 189: 113-130. The cloned gene fragment codes for a methionine at the N-terminus of the CBD, but the rest of the CBD aa sequence corresponds to residues 28 to 189 of CbpA. The protein fragment has a molecular weight of 17634. The insertion was verified by DNA sequencing. CBD protein was produced by E. coli BL21 (DE3) cells harboring PET-CBD. After the addition of IPTG, this host strain produces T7 RNA polymerase, which recognizes the T7 promotor in the pET vector. The cbd gene fragment was under the control of this inducible promotor, and CBD protein was synthesized in large amounts after induction (FIG. 3). After a four h production period, the soluble extract from the lysed cells contained only small amounts of CBD protein, while most was found in the insoluble fraction. This protein was readily soluble in concentrated guanidine hydrochloride, and was renatured by slow dilution into TEDG buffer. It was found that protein prepared in this fashion binds to AVICIL.RTM. (microcrystalline cellulose), verifying the putative CBD. Although this fraction is mostly CBD protein, the assays described require the protein to be highly pure. This purity is provided by a single cellulose-affinity binding step, as described in the Section 7.1.1. The affinity-purified CBD protein appears on acrylamide gels as a single band when stained with Coomasie brilliant blue. Approximately 70 mg of CBD protein can be recovered from the cells harvested from a 1 l culture.

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L5: Entry 1 of 1 File: USPT Sep 17, 2002

DOCUMENT-IDENTIFIER: US 6451995 B1
TITLE: Single chain FV polynucleotide or peptide constructs of anti-ganglioside GD2 antibodies, cells expressing same and related methods

Abstract Text (1):
Recombinant antibody constructs comprise the variable regions of the heavy and light chains of anti-G.sub.D2 antibodies. These antibody constructs may be coupled to a label such as a radiolabel or to a protein such as streptavidin or pro-drug converting enzymes for use in imaging or therapeutic applications. The antibody constructs may also be transduced into T cells to produce populations of T cells which target G.sub.D2 -producing tumor cells.

US Patent No. (1):
6451995

Brief Summary Text (18):
The antibodies of the present invention are recombinant antibody constructs comprising the variable regions of the heavy and light chains of anti-G.sub.D2 antibodies. These antibody constructs may be coupled to a label such as a radiolabel or to a protein such as streptavidin or pro-drug converting enzymes for use in imaging or therapeutic applications. The antibody constructs may also be transduced into T cells to produce populations of T cells which target G.sub.D2 -producing tumor cells.

Detailed Description Text (4):
In order to increase the avidity of the scFv, we have synthesized two scFv variants: (1) Cysteine residue at the carboxyl terminal of the scFv for dimerization (5FpoMCH of Table 2 and 3GpoMCH of Table 3): Free sulhydryl groups are blocked by acetylation and the monomer separated from the dimer by size-exclusion chromatography FPLC on Sephadex HR75 (Pharmacia). (2) Streptavidin at the carboxyl end for dimerization and tetramerization (5FpoStMCH of table 1 and 3GpoStMCH of table 2): Streptavidin is a homo-tetrameric protein that binds one biotin molecule per subunit with a very high affinity (Kd=4.times.10-14). scFv-strep fusion proteins are expected to form tetramers with both antigen- and biotin-binding activity. They are expected to be stable over a wide range of pH and range of physiologic temperatures.

Detailed Description Text (5):
The 5F11-scFv, 3G6-scFv, 5F11-scFv-streptavidin, 3G6-scFv-streptavidin DNA sequences are shown below, with the linker sequences between the scFv and the streptavidin shown in lower case letters.

Detailed Description Text (8):
5F11-scFv-Streptavidin (SEQ ID NO. 3)
CAGGTGAAACTGCAGCAGTCAGGACCTGAACTGGTGNAGCCTGGGGCTTCAG
TGAAGATATCCTGCAAGACTTCTGGANACAAATTCACTGAATACACCATGCAC
TGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGAGGTATTAAT
CCTAACAATGGTGGTACTAACTACAAGCAGAAGTTCAAGGGCAAGGCCACAT
TGACTGTAGACAAGTCCTCCAGCACAGCCTACATGGAGCTCCGCAGCCTGAC
ATCTGAGGATTCTGCAGTCTATTACTGTGCAAGAGATACTACGGTCCCGTTTG
CTTACTGGGTCCAAGGGACCACGGTCACCGTCTCCTCAGGTGGAGGCGGTTC
AGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGACATCGAGCTCACTCAGTCT
CCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCACCATGACCTGCAGTG

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GCAGCTCAAGTATAAGTTACATGCACTGGTACCAGCAGAAGCCTGTCACCTCC
CCCAAAAGATGGATTTATGACACATCCAAACTGGCTTCTGGAGTCCCTGCTCG
CTTCAGTGGCAGTGGGTCTGGGACCTCTTATTCTCTCACAATCAGCAGCATGG
AGGCTGTAGATGCTGCCACTTATTACTGCCATCAGCGGAGTAGTTACCCGCTC
ACGTTCGGTGCTGGGACACAGTTGGAAATAAAACGGgcggccgctggatccggtgctgct
GAAGCAGGTATCACCGGCACCTGGTACAACCAGCTCGGCTCGACCTTCATCGT
GACCGCGGGCGCCGACGGCGCCCTGACCGGAACCTACGAGTCGGCCGTCGG
CAACGCCGAGAGCCGCTACGTCCTGACCGGTCGTTACGACAGCGCCCCGGCC
ACCGACGGCAGCGGCACCGCCCTCGGTTGGACGGTGGCCTGGAAGAATAACT
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CGAGGCGAGGATCAACACCCAGTGGCTGCTGACCTCCGGCACAACCGAGGCC
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Detailed Description Text (9):
3G6-scFv-streptavidin (SEQ ID NO. 4)
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Detailed Description Text (12):
Metal chelation to scFv can also be accomplished via the streptavidin protein. The rationale of pretargeting using scFv-streptavidin fusion proteins in radioimmunotherapy are 5-fold: (a) Large amounts of scFv can be used to saturate G.sub.D2 sites in vivo, without the accompanying blood and tissue toxicity from radioisotope, (b) radiolabel is injected at the time when the tumor-nontumor ratio of scFv is maximal, (c) a radiolabeled ligand is chosen such that it binds with high affinity (e.g. 111I-biotin binding to streptavidin) with fast blood-clearance, (d) a ligand construction where the isotope can be modified to optimize microdosimetry (e.g. SHNH-biotin) (e) the scFv-streptavidin is a homo-tetramer, as such the antigen binding avidity is greatly amplified especially for high-density antigens (e.g. G.sub.D2 on neuroblastoma). scFv-strep fusion proteins for both 5F11 and 3G6 have been made and purified. Both in vitro and in vivo studies are being carried out to test the concept of pretargeting, where scFv-strep is first allowed to bind (or target) to G.sub.D2 -positive tumors through the scFv. After the excess or nonbinding scFv-strep is washed off (or cleared from the body), a radiolabeled-biotin ligand is allowed to bind to the streptavidin moiety. Different radiolabels can be coupled to biotin using SHNH (.sup.99m Tc) or DTPA (.sup.111 In or yttrium).

Detailed Description Text (14):
The scFv and scFv-streptavidin of the invention are also useful in a number of therapeutic applications, which is turn form aspects of the present invention. In general, these approaches involve administration of scFV coupled to a therapeutic or pre-therapeutic moiety. For example, as shown in FIG. 1, ScFv-streptavidin (streptavidin being the pre-therapeutic moiety) is introduced into an organism suspected of harboring G.sub.D2 expressing cells, where it binds to any such cells

present. A therapeutic agent (X) bound to biotin is then introduced. Binding of the biotin the streptavidin results in localization of the chemotherapeutic agent X at the site of the G.sub.D2 producing cells. Other pre-therapeutic moieties include pro-drug converting enzymes. Directly therapeutic moieties such as toxins can also be used.

Detailed Description Text (16):
The scFv or scFv-streptavidin can be incorporated in a fusion protein with therapeutic agents such as toxins or pro-drug converting enzymes, can be incorporated in a fusion protein with CD8 to facilitate the formation of G.sub.D2 -targeted lymphocytes, or can be coupled to viral coat proteins superantigen (SEA) to facilitate targeting of G.sub.D2 producing cells.

Detailed Description Text (17):
Direct conjugation of scFv or scFv-streptavidin to toxin replaces the cell-binding domain of natural toxins with the scFv, which serves as a tumor binding domain specific to G.sub.D2 expressing cells. ScFv-ricin-A-chain and scFv-pseudomonas toxin have been successfully constructed for other scFv. This coupling is advantageously performed at the DNA level, not at the protein level. For example, when the fusion protein of the heavy chain, the light chain and the linker is created by overlap PCR extension, a DNA coding for the toxin can also included, and then expressed along with the scFv.

Detailed Description Text (18):
scFv and scFv-streptavidin can also be usefully combined in a fusion protein with CD8. scFv-CD8 constructs can be transfected through retroviral vector into human and mouse lymphocytes. Since these scFv are permanently integrated into the cellular genome, these lymphocytes express scFv on their cell surface and through the CD8 cytoplasmic domain become activated upon antigen binding. scFv facilitates the homing of these cells to tumor sites, thus being effective in promoting both the localization and killing of tumors. With a suicide gene, thymidine kinase, also transfected, these cells can now be turned on and off as needed.

Detailed Description Text (19):
scFv-enzyme and scFv-enzyme-streptavidin conjugates can be used to provide targeted drug therapy using a technique known as ADEPT (antibody directed enzyme prodrug-therapy). Suitable enzymes for this technique include carboxypeptidase G2, alkaline phosphatase, and .beta.-Lactamase. A prodrug derivative (e.g. cephalosporin derivative of doxo20) becomes activated to the active agent by the enzyme (beta-lactamase) targeted to the tumor by the scFv. Thus tumor cells are exposed to a high local concentration (up to 10-fold higher than blood/tissue levels) of specific chemotherapeutic agents.

Detailed Description Text (20):
Integration of scFv (with or without streptavidin) into viral coat proteins can be used to retarget these viruses in vivo. These viruses include adenovirus, retrovirus and herpes virus.

Detailed Description Text (21):
Superantigen (SEA) can stimulate T cells without the requirement of MHC.21 ScFv-SEA and scFv-streptavidin-SEA can target T cells to lyse antigen-positive MHC-class II-negative human tumor cells. SEA has been cloned (Betley et al: J. Bacteriology 170: 34-41, 1988) and the cDNA is available for making fusion proteins.

Detailed Description Text (28):
For construction of the 5FpoStMCH vector which contains the 5F11-scFV-streptavidin plasmid DNA, plasmid DNA from the 5F11-scFv in pCantab 5E vector (Pharmacia Biotech) was purified and amplified by PCR using two specially designed primers S6 and 318s. S6 contains a NotI restriction site and 318s contains a PvuII restriction site so that amplified DNA can be restriction digested and inserted in the pSTE vector (Dr. Dubel, German Cancer Center). The resulting vector 5FpoStMCH is the 5F11-scFv-streptavidin construct. The streptavidin was digested with BamHI, leaving the scFV 5FpoMCH.

Detailed Description Text (34):
In constructing 3G6-scFv, the orientation VH-VL did not produce a functional scFV. Therefore the orientation VL-VH was used. cDNA of VH and VL of 3G6 hybridoma were

linked through a custom built linker and inserted into the pHEN vector (DR. Greg Winter). NcoI and NotI restriction sites were built into the VH and VL linkers so that the scFV can be digested with these enzymes for insertion in the pSTE vector. Clone 7 was chosen and called 3GpoStMCH. Digestion of the streptavidin position of the gene left behind 3G6-scFv, now called 3GpoMCH. The following references are cited above, and are incorporated herein by reference. 1. Rodden F A, Wiegandt H, Bauer B L: Gangliosides: the relevance of current research to neurosurgery. J Neurosurg 74:606-619, 1991 2. Berra B, Gaini S M, Riboni L: Correlation between ganglioside distribution and histological grading of human astrocytoma. Int J Cancer 36:363-366, 1985 3. Traylor T D, Hogan E L: Gangliosides of human cerebral astrocytomas. J Neurochem 34:126-131, 1980 4. Ye J N, Cheung N K V: A novel O-acetylated ganglioside detected by anti-G.sub.D2 monoclonal antibodies. Int J Cancer 50:197-201, 1992 5. Wikstrand C J, Fredman P, Svennerholm L, et al: Expression of gangliosides GM2, G.sub.D2, GD3, 3'-sioLM1, and 3',6' isoLD1 in CNS malignancies as defined by epitope-characterized monoclonal antibodies (Mabs). 9th International Conference on Brain Tumors Research and Therapy 1991 (abstract) 6. Longee D C, Wikstrand C J, Mansson J E, et al: Disialoganglioside G.sub.D2 in human neuroectodermal tumor cell lines and gliomas. Acta-Neuropathology (Berl) 82:45-54,1991 7. Hoon D S, Banez M, Okun E, et al: Modulation of human melanoma cells by interleukin-4 and in combination with gamma-interferon or alpha-tumor necrosis factor. Cancer Res 51:2002-2008, 1991 8. Arbit E, Yeh S J, Cheung N K, Larson S M: Quantitative Immunoimaging of gliomas in humans with anti-ganglioside monoclonal antibodies. J Neurosurg 76:399a, 1991 9. Saito M, Yu R K, Cheung N K V: Ganglioside G.sub.D2 specificity of monoclonal antibodies to human neuroblastoma cell. Biochem Biophys Res Comm 127:14, 1985 10. Lammie G A, Cheung N K V, Gerald W, et al: Ganglioside G.sub.D2 expression in the human nervous system and in neuroblastomas--an immunohistochemical study. Int J Oncol 3:909-915, 1993 11. Sariola H, Terava H, Rapola J, Saarinen U M: Cell-Surface Ganglioside G.sub.D2 in the Immunohistochemical Detection and Differential Diagnosis of Neuroblastoma. AJCP 96:248-252, 1991 12. Saarinen U M, Sariola H, Hovi L: Recurrent Disseminated Retinoblastoma Treated by High-dose Chemotherapy, Total Body Irradiation, and Autologous Bone Marrow Rescue. Am J Pediatr Hematol/Oncol 13:315-319, 1991 13. Heiner J, Miraldi F D, Kallick S, et al: In vivo targeting of G.sub.D2 specific monoclonal antibody in human osteogenic sarcoma xenografts. Cancer Res 47:5377-5381, 1987 14. Chang H R, Cordon-Cardo C, Houghton A N, et al: Expression of disialogangliosides G.sub.D2 and GD3 by human soft tissue sarcomas. Cancer 70:633-638, 1992 15. Cheung N K, Neely J E, Landmeier B, et al: Targeting of ganglioside G.sub.D2 monoclonal antibody to neuroblastoma. J Nucl Med 28:1577-1583, 1987 16. Yeh S D, Larson S M, Burch L, et al: Radioimmunodetection of neuroblastoma with iodine-131-3F8: Correlation with biopsy, iodine-131-Metaiodobenzylguanidine (MIBG) and standard diagnostic modalities. J Nucl Med 32:769-776, 1991 17. Miraldi F D, Nelson A D, Kraly C, et al: Diagnostic imaging of human neuroblastoma with radiolabeled antibody. Radiology 161:413-418, 1986 18. Grant S C, Kostakoglu L, Kris M G, et al: Imaging of small cell lung carcinoma with the monoclonal antibody 3F8. Proc Am Soc Clin Oncol 10:265, 1991 (abstract) 19. Yeh S D J, Casper E S, Cheung N K V, et al: Radioimmunoimaging of soft-tissue sarcoma with an anti-ganglioside monoclonal antibody 3F8. 5th Asia & Oceania Cong of Nucl Med & Biol Proceedings: 104, 1992 20. Svenson H P, Vrudhula V M, Emswiler J E, et al: In Vitro and In Vivo Activities of a Doxorubicin Prodrug in Combination with Monoclonal Antibody .beta.-Lactamase Conjugates. Cancer Res 55:2357-65, 1995 21. Dohisten M, Abrahmsen L, Bjork P, et al: Monoclonal antibody-superantigen fusion proteins:Tumor-specific agents for T-cell-based tumor therapy. Proc Natl Acad Sci (USA) 91:8945-8949, 1994 22. Dhingra K, Fritsch H, Murray J L, et al: Phase I Clinical and Pharmacological Study of Suppression of Human Antimouse Antibody Response to Monoclonal antibody L6 by Deosxysspergualin. Cancer Res 55:3060-67, 1995 23. Wnag C-Y, Huang L: p-H-sensitive immunoliposomes mediate target-cell-specific delivery and controlled expression of a foreign gene in mouse. Proc Natl Acad Sci (USA) 84:7851-55, 1987 24. Vieweg J, Boczkowski D, Roberson K M, et al: Efficient Gene Transfer with Adeno-associated Virus-based Plasmids Complexed to Cationic Liposomes for Gene Therapy of Human Prostate Cancer. Cancer Res 55:2366-2372, 1995 25. Lorimer I A J, Wikstrand C J, Batra S K, et al: Immunotixins That Target an Oncogenic Mutant Euidermal Growth Factor Receptor Expressed in Human Tumors. Clin Can Res 1:859-64, 1995

Detailed Description Paragraph Table (3):
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CLAIMS:

4. The recombinant polynucleotide of claim 3, wherein the additional protein is streptavidin.

7. The recombinant polynucleotide of claim 6, wherein the additional protein is streptavidin.

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L4: Entry 14 of 120 File: USPT Sep 17, 2002

DOCUMENT-IDENTIFIER: US 6451995 B1
TITLE: Single chain FV polynucleotide or peptide constructs of anti-ganglioside GD2 antibodies, cells expressing same and related methods

Application Filing Year (1):
1998

Detailed Description Text (27):
The selected phage was used to reinfect E coli XL1-Blue cells. Clones were grown in 2XYT medium containing ampicillin (100 ug/ml) and 1% glucose at 30.degree. C. until an OD600 of 0.5 was obtained. Expression of ScFv antibody was induced by changing to a medium containing 100 uM IPTG and incubating overnight at 300.degree. C. The supernatant obtained from the medium by centrifugation was directly added to a plate coated with GD2. The pellet was resuspended in PBD containing 1 mM EDTA and incubated on ice for 10 minutes. The periplasmic soluble antibody was collected by centrifugation again and added to the plate. After incubating at 37.degree. C. for 32 hours, anti-E Tag antibody (Pharmacia Biotech) was used to specifically screen the binding of the ScFv fragment.

Detailed Description Text (34):
In constructing 3G6-scFv, the orientation VH-VL did not produce a functional scFV. Therefore the orientation VL-VH was used. cDNA of VH and VL of 3G6 hybridoma were linked through a custom built linker and inserted into the pHEN vector (DR. Greg Winter). NcoI and NotI restriction sites were built into the VH and VL linkers so that the scFV can be digested with these enzymes for insertion in the pSTE vector. Clone 7 was chosen and called 3GpoStMCH. Digestion of the streptavidin position of the gene left behind 3G6-scFv, now called 3GpoMCH. The following references are cited above, and are incorporated herein by reference. 1. Rodden F A, Wiegandt H, Bauer B L: Gangliosides: the relevance of current research to neurosurgery. J Neurosurg 74:606-619, 1991 2. Berra B, Gaini S M, Riboni L: Correlation between ganglioside distribution and histological grading of human astrocytoma. Int J Cancer 36:363-366, 1985 3. Traylor T D, Hogan E L: Gangliosides of human cerebral astrocytomas. J Neurochem 34:126-131, 1980 4. Ye J N, Cheung N K V: A novel O-acetylated ganglioside detected by anti-G.sub.D2 monoclonal antibodies. Int J Cancer 50:197-201, 1992 5. Wikstrand C J, Fredman P, Svennerholm L, et al: Expression of gangliosides GM2, G.sub.D2, GD3, 3'-sioLM1, and 3',6' isoLD1 in CNS malignancies as defined by epitope-characterized monoclonal antibodies (Mabs). 9th International Conference on Brain Tumors Research and Therapy 1991 (abstract) 6. Longee D C, Wikstrand C J, Mansson J E, et al: Disialoganglioside G.sub.D2 in human neuroectodermal tumor cell lines and gliomas. Acta-Neuropathology (Berl) 82:45-54,1991 7. Hoon D S, Banez M, Okun E, et al: Modulation of human melanoma cells by interleukin-4 and in combination with gamma-interferon or alpha-tumor necrosis factor. Cancer Res 51:2002-2008, 1991 8. Arbit E, Yeh S J, Cheung N K, Larson S M: Quantitative Immunoimaging of gliomas in humans with anti-ganglioside monoclonal antibodies. J Neurosurg 76:399a, 1991 9. Saito M, Yu R K, Cheung N K V: Ganglioside G.sub.D2 specificity of monoclonal antibodies to human neuroblastoma cell. Biochem Biophys Res Comm 127:14, 1985 10. Lammie G A, Cheung N K V, Gerald W, et al: Ganglioside G.sub.D2 expression in the human nervous system and in neuroblastomas--an immunohistochemical study. lnt J Oncol 3:909-915, 1993 11. Sariola H, Terava H, Rapola J, Saarinen U M: Cell-Surface Ganglioside G.sub.D2 in the Immunohistochemical Detection and Differential Diagnosis of Neuroblastoma. AJCP 96:248-252, 1991 12. Saarinen U M, Sariola H, Hovi L: Recurrent Disseminated Retinoblastoma Treated by High-dose Chemotherapy, Total Body Irradiation, and Autologous Bone Marrow Rescue. Am J Pediatr Hematol/Oncol 13:315-319, 1991 13. Heiner

J, Miraldi F D, Kallick S, et al: In vivo targeting of G.sub.D2 specific monoclonal antibody in human osteogenic sarcoma xenografts. Cancer Res 47:5377-5381, 1987 14. Chang H R, Cordon-Cardo C, Houghton A N, et al: Expression of disialogangliosides G.sub.D2 and GD3 by human soft tissue sarcomas. Cancer 70:633-638, 1992 15. Cheung N K, Neely J E, Landmeier B, et al: Targeting of ganglioside G.sub.D2 monoclonal antibody to neuroblastoma. J Nucl Med 28:1577-1583, 1987 16. Yeh S D, Larson S M, Burch L, et al: Radioimmunodetection of neuroblastoma with iodine-131-3F8: Correlation with biopsy, iodine-131-Metaiodobenzylguanidine (MIBG) and standard diagnostic modalities. J Nucl Med 32:769-776, 1991 17. Miraldi F D, Nelson A D, Kraly C, et al: Diagnostic imaging of human neuroblastoma with radiolabeled antibody. Radiology 161:413-418, 1986 18. Grant S C, Kostakoglu L, Kris M G, et al: Imaging of small cell lung carcinoma with the monoclonal antibody 3F8. Proc Am Soc Clin Oncol 10:265, 1991 (abstract) 19. Yeh S D J, Casper E S, Cheung N K V, et al: Radioimmunoimaging of soft-tissue sarcoma with an anti-ganglioside monoclonal antibody 3F8. 5th Asia & Oceania Cong of Nucl Med & Biol Proceedings: 104, 1992 20. Svenson H P, Vrudhula V M, Emswiler J E, et al: In Vitro and In Vivo Activities of a Doxorubicin Prodrug in Combination with Monoclonal Antibody .beta.-Lactamase Conjugates. Cancer Res 55:2357-65, 1995 21. Dohisten M, Abrahmsen L, Bjork P, et al: Monoclonal antibody-superantigen fusion proteins:Tumor-specific agents for T-cell-based tumor therapy. Proc Natl Acad Sci (USA) 91:8945-8949, 1994 22. Dhingra K, Fritsch H, Murray J L, et al: Phase I Clinical and Pharmacological Study of Suppression of Human Antimouse Antibody Response to Monoclonal antibody L6 by Deosxysspergualin. Cancer Res 55:3060-67, 1995 23. Wnag C-Y, Huang L: p-H-sensitive immunoliposomes mediate target-cell-specific delivery and controlled expression of a foreign gene in mouse. Proc Natl Acad Sci (USA) 84:7851-55, 1987 24. Vieweg J, Boczkowski D, Roberson K M, et al: Efficient Gene Transfer with Adeno-associated Virus-based Plasmids Complexed to Cationic Liposomes for Gene Therapy of Human Prostate Cancer. Cancer Res 55:2366-2372, 1995 25. Lorimer I A J, Wikstrand C J, Batra S K, et al: Immunotixins That Target an Oncogenic Mutant Euidermal Growth Factor Receptor Expressed in Human Tumors. Clin Can Res 1:859-64, 1995

L4: Entry 75 of 120 File: USPT Aug 17, 1999

DOCUMENT-IDENTIFIER: US 5939531 A
TITLE: Recombinant antibodies specific for a growth factor receptor

Application Filing Year (1):
1995

Brief Summary Text (12):
The preferred recombinant antibody of the invention is a single-chain antibody wherein the heavy chain variable domain and the light chain variable domain are linked by way of a spacer group, preferably a peptide. Most preferred is a single-chain antibody wherein the heavy chain variable domain is located at the N-terminus of the recombinant antibody. The single-chain recombinant antibody may further comprise an effector molecule and/or signal sequences facilitating the processing of the antibody by the host cell in which it is prepared. Effector molecules considered are those useful for diagnostic or therapeutic purposes, for example enzymes causing a detectable reaction, e.g. phosphatase, such as alkaline phosphatase from E. coli or mamalian alkaline phosphatase, e.g. bovine alkaline phosphatase, horseradish peroxidase, .beta.-D-galactosidase, glucose oxidase, glucoamylase, carbonic anhydrase, acetylcholinesterase, lysozyme, malate dehydrogenase or glucose-6-phosphate, a peptide having particular binding properties, e.g. streptavidin from Streptomyces avidinii strongly binding to biotin, or enzymes, toxins or other drugs attacking the cells to which the antibody is bound, e.g. a protease, a cytolysin or an exotoxin, for example ricin A, diphtheria toxin A, or Pseudomonas exotoxin. In the following a single-chain recombinant antibody further comprising an effector molecule is referred to as fusion protein or intended to be within the meaning of the terms "single chain (recombinant) antibody" or "recombinant antibody", if appropriate.

Detailed Description Text (62):
In this construct the Fv single-chain antibody of FRP5, genetically fused to the alkaline phosphatase phoA, can be expressed in E. coli following induction with IPTG. The recombinant protein carries the E. coli outer membrane protein A (ompA) signal sequence at the N terminus (encoded by the pINIII-ompA-Hind vector) to facilitate secretion of the protein into the periplasmic space of E. coli expressor cells.

Detailed Description Text (67):
7.1 Isolation of Fv(FRP5)-phoA from the periplasmic proteins of CC118/pWW616: The bacterial pellet is suspended in 10 ml TES buffer (0.2 M Tris-HCl, pH 8.0, 0.5 mM EDTA, 0.5 M sucrose) and kept on ice for 10 min. After centrifugation at 4.degree. C. for 10 min at 5000 rpm in a Heraeus minifuge, the supernatant is discarded and the washed pellet is suspended in 15 ml ice-cold TES, diluted (1:4) with water. The cells are kept on ice for 30 min and recentrifuged as above. The supernatant containing periplasmic proteins is recentrifuged at 45,000.times.g for 15 min in a Beckman TL100 ultracentrifuge. The periplasmic extract is concentrated in an Amersham ultrafiltration unit through a YM10 membrane to a final volume of 2 ml. Following fivefold dilutions with PBS and reconcentration through the YM10 membrane five times, the 1:4 diluted TES buffer of the periplasmic extract is exchanged with PBS. NaN.sub.3 and protease inhibitors are added to the periplasmic proteins (2 ml in PBS) to the final concentration of 0.02% NaN.sub.3, 0.1 mM PMSF, 2 .mu.g/ml aprotinin, 1 .mu.g/ml leupeptin, and 1 .mu.g/ml pepstatin. The periplasmic extract is stored at 4.degree. C.

Detailed Description Text (73):
8.1.2 Pretreatment of Fv(FRP5)-phoA: Alkaline phosphatase phoA from E. coli must be

dimerized to be enzymatically active. In the periplasm of E. coli natural phoA is dimerized, i.e. two molecules of phoA are held together by two Zn.sup.2+ ions. The Fv(FRP5)-phoA is also produced as a dimer in E. coli. To increase binding of Fv(FRP5)-phoA to the antigen, the dimers are monomerized by adding EGTA to the solution. This step removes Zn.sup.2+ from the solution. Monomerized phosphatase can be re-dimerized by the addition of Zn.sup.2+. EGTA is added to a final concentration of 5 mM to 200 .mu.l of 40.times.concentrated supernatant or periplasmic proteins from CC118/pWW616 (see above). The solution is incubated at 37.degree. C. for 1 h just before use in the immunoassay.

Detailed Description Text (78):
9.1 Preparation of periplasmic extract: Plasmid pWW616 is transformed into the phoA negative E. coli strain CC118 according to standard procedures (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982). A single colony is picked and grown overnight in LB medium containing 70 .mu.g/ml ampicillin. The overnight culture is diluted 1:10 in fresh LB medium containing ampicillin and grown at 37.degree. C. to an OD.sub.550 of 0.1. At this point expression of the Fv(FRP5)-phoA gene is induced by the addition of IPTG to a final concentration of 2 mM, and the cells are grown for an additional 1.5 to 2 h. The cells are harvested by centrifugation and treated with a mild osmotic shock which releases the periplasmatic proteins into the supernatant. The proteins are concentrated in an Amersham ultrafiltration unit through a YM10 membrane.

Detailed Description Text (84):
10.2 Pretreatment of Fv(FRP5)-phoA: Since the dimer of the Fv(FRP5)-phoA as obtained from the E. coli periplasm does not bind optimally to the c-erbB-2 antigen, it is first monomerized. This is accomplished by treating the solution of Fv(FRP5)-phoA for 1 h at 37.degree. C. with EGTA at a final concentration of 5 mM. This treatment chelates the Zn.sup.2+ ions which are important for maintaining the dimeric structure of Fv(FRP5)-phoA.

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L4: Entry 107 of 120 File: USPT Nov 5, 1996

DOCUMENT-IDENTIFIER: US 5571894 A
TITLE: Recombinant antibodies specific for a growth factor receptor

Application Filing Year (1):
1994

Brief Summary Text (12):
The preferred recombinant antibody of the invention is a single-chain antibody wherein the heavy chain variable domain and the light chain variable domain are linked by way of a spacer group, preferably a peptide. Most preferred is a single-chain antibody wherein the heavy chain variable domain is located at the N-terminus of the recombinant antibody. The single-chain recombinant antibody may further comprise an effector molecule and/or signal sequences facilitating the processing of the antibody by the host cell in which it is prepared. Effector molecules considered are those useful for diagnostic or therapeutic purposes, for example enzymes causing a detectable reaction, e.g. phosphatase, such as alkaline phosphatase from E. coli or mamalian alkaline phosphatase, e.g. bovine alkaline phosphatase, horseradish peroxidase, .beta.-D-galactosidase, glucose oxidase, glucoamylase, carbonic anhydrase, acetylcholinesterase, lysozyme, malate dehydrogenase or glucose-6-phosphate, a peptide having particular binding properties, e.g. streptavidin from Streptomyces avidinii strongly binding to biotin, or enzymes, toxins or other drugs attacking the cells to which the antibody is bound, e.g. a protease, a cytolysin or an exotoxin, for example ricin A, diphtheria toxin A, or Pseudomonas exotoxin. In the following a single-chain recombinant antibody further comprising an effector molecule is referred to as fusion protein or intended to be within the meaning of the terms "single chain (recombinant) antibody" or "recombinant antibody", if appropriate.

Detailed Description Text (54):
In this construct the Fv single-chain antibody of FRP5, genetically fused to the alkaline phosphatase phoA, can be expressed in E. coli following induction with IPTG. The recombinant protein carries the E. coli outer membrane protein A (ompA) signal sequence at the N terminus (encoded by the pINIII-ompA-Hind vector) to facilitate secretion of the protein into the periplasmic space of E. coli expressor cells.

Detailed Description Text (59):
7.1 Isolation of Fv(FRP5)-phoA from the periplasmic proteins of CC118/pWW616: The bacterial pellet is suspended in 10 ml TES buffer (0.2M Tris-HCl, pH 8.0, 0.5 mM EDTA, 0.5M sucrose) and kept on ice for 10 min. After centrifugation at 4.degree. C. for 10 min at 5000 rpm in a Heraeus minifuge, the supernatant is discarded and the washed pellet is suspended in 15 ml ice-cold TES, diluted (1:4) with water. The cells are kept on ice for 30 min and recentrifuged as above. The supernatant containing periplasmic proteins is recentrifuged at 45,000.times.g for 15 min in a Beckman TL100 ultracentrifuge. The periplasmic extract is concentrated in an Amersham ultrafiltration unit through a YM10 membrane to a final volume of 2 ml. Following fivefold dilutions with PBS and reconcentration through the YM10 membrane five times, the 1:4 diluted TES buffer of the periplasmic extract is exchanged with PBS. NaN.sub.3 and protease inhibitors are added to the periplasmic proteins (2 ml in PBS) to the final concentration of 0.02% NaN.sub.3, 0.1 mM PMSF, 2 .mu.g/ml aprotinin, 1 .mu.g/ml leupeptin, and 1 .mu.g/ml pepstalin. The periplasmic extract is stored at 4.degree. C.

Detailed Description Text (65):
8.1.2 Pretreatment of Fv(FRP5)-phoA: Alkaline phosphatase phoA from E. coli must be

dimerized to be enzymatically active. In the periplasm of E. coli natural phoA is dimerized, i.e. two molecules of phoA are held together by two Zn.sup.2+ ions. The Fv(FRP5)-phoA is also produced as a dimer in E. coli. To increase binding of Fv(FRP5)-phoA to the antigen, the dimers are monomerized by adding EGTA to the solution. This step removes Zn.sup.2+ from the solution. Monomerized phosphatase can be re-dimerized by the addition of Zn.sup.2+. EGTA is added to a final concentration of 5 mM to 200 .mu.l of 40.times.concentrated supernatant or periplasmic proteins from CC118/pWW616 (see above). The solution is incubated at 37.degree. C. for 1 h just before use in the immunoassay.

Detailed Description Text (70):
9.1 Preparation of periplasmic extract: Plasmid pWW616 is transformed into the phoA negative E. coli strain CC118 according to standard procedures (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982). A single colony is picked and grown overnight in LB medium containing 70 .mu.g/ml ampicillin. The overnight culture is diluted 1:10 in fresh LB medium containing ampicillin and grown at 37.degree. C. to an OD.sub.550 of 0.1. At this point expression of the Fv(FRP5)-phoA gene is induced by the addition of IPTG to a final concentration of 2 mM, and the cells are grown for an additional 1.5 to 2 h. The cells are harvested by centrifugation and treated with a mild osmotic shock which releases the periplasmatic proteins into the supernatant. The proteins are concentrated in an Amersham ultrafiltration unit through a YM10 membrane.

Detailed Description Text (76):
10.2 Pretreatment of Fv(FRP5)-phoA: Since the dimer of the Fv(FRP5)-phoA as obtained from the E. coli periplasm does not bind optimally to the c-erbB-2 antigen, it is first monomerized. This is accomplished by treating the solution of Fv(FRP5)-phoA for 1 h at 37.degree. C. with EGTA at a final concentration of 5 mM. This treatment chelates the Zn.sup.2+ ions which are important for maintaining the dimeric structure of Fv(FRP5)-phoA.

☐ 1: Protein Expr Purif. 1998 Nov;14(2):192-6. Related Articles, Links

Production of a soluble and functional recombinant streptavidin in Escherichia coli.

Gallizia A, de Lalla C, Nardone E, Santambrogio P, Brandazza A, Sidoli A, Arosio P.

Dibit, Department of Biological and Technological Research, San Raffaele Scientific Institute, Milan, 20132, Italy.

The cDNA for streptavidin (residues 15-159) was subcloned into an expression vector in fusion at the N-terminus with the T7-tag (12 residues). Conditions were found to express the protein in Escherichia coli in a soluble, assembled, and active form. The protein was purified in two simple steps which involved heating at 75 degreesC and affinity chromatography on iminobiotin agarose. The purified protein was obtained in yields of 70 mg per liter of bacterial culture. Electron spray mass spectrometry analysis showed that the recombinant streptavidin had the expected molecular mass without covalent modifications. ELISA and surface plasmon resonance analyses showed it to be functionally analogous to the natural streptavidin. This appears to be an improvement over the reported methods of recombinant streptavidin production which involve protein renaturation or the use of eukaryotic expression systems. Copyright 1998 Academic Press.

PMID: 9790881 [PubMed - indexed for MEDLINE]

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1: Mol Biotechnol. 1999 Aug;12(1):25-34. Related Articles, Links

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Expression and secretion of proteins in E. coli.

Pines O, Inouye M.

Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel.

This review outlines approaches to the cloning and expression of proteins in Escherichia coli. The expression vectors described here (pIN-III derivatives) utilize the strong lipoprotein promoter, which is controlled by the lac-UV5 promoter-operator. These vectors provide the means for targeting a protein to any of the four subcellular compartments of the bacterial cell: cytoplasm, cytoplasmic membrane, periplasm, and outer membrane. Of particular importance is that secretion of proteins into the E. coli periplasm (using the OmpA signal peptide) is applicable for the production of both prokaryotic and eukaryotic proteins thereby enhancing protein activity and stability.

Publication Types:
- Review
- Review, Tutorial

PMID: 10554771 [PubMed - indexed for MEDLINE]

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1: Appl Microbiol Biotechnol. 1993 Mar;38(6):770-5. Related Articles, Links

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High-level production and secretion of a mouse-human chimeric Fab fragment with specificity to human carcino embryonic antigen in Escherichia coli.

Shibui T, Munakata K, Matsumoto R, Ohta K, Matsushima R, Morimoto Y, Nagahari K.

Biosciences Laboratory, Mitsubishi Kasei Corporation, Kanagawa, Japan.

A high-level secretion system for the production of mouse-human chimeric antibody 21B2 (MHC 21B2) Fab fragment specific for human carcino embryonic antigen (hCEA) in Escherichia coli has been constructed. The genes encoding a light chain and an Fd fragment (a variable region and the CH1 domain of a heavy chain) of a mouse-human chimeric antibody were directly fused to the signal peptide of the E. coli ompF gene sequence. E. coli cells containing expression vectors in which each of the two genes are located downstream of a separate tac promoter were able to secrete the light chain and Fd fragment as two of their major cellular proteins. The signal peptides were efficiently removed from the primary products by post-translational processing, although they formed insoluble aggregates, possibly in the periplasm. In high-cell-density culture experiments using a jar fermentor, the amount of light chain and Fd fragment produced was at levels of up to 2.88 g/l and 1.28 g/l culture, respectively. By optimizing the conditions that encourage correct folding, formation of disulphide bonds, and association of the light chain with the Fd fragment, we have established a procedure that can purify, re-fold, and combine aggregated products to electrophoretically homogeneous Fab fragment with a yield of approximately 47%. Fab fragment produced in this manner shows essentially the same antigen-binding activity and specificity to hCEA as the parental mouse antibody 21B2 (MoAb 21B2).

PMID: 7763534 [PubMed - indexed for MEDLINE]

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1: Appl Microbiol Biotechnol. 1992 Jun;37(3):352-7. Related Articles, Links

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Secretion of a functional Fab fragment in Escherichia coli and the influence of culture conditions.

Shibui T, Nagahari K.

Biosciences Laboratory, Mitsubishi Kasei Corporation, Kanagawa, Japan.

Genes encoding a light chain and an Fd region (a variable region and a CH1 domain of a heavy chain) of a mouse-human chimeric antibody with specificity for human carcinoembryonic antigen (CEA) were fused to a DNA segment coding for the signal peptide of Escherichia coli ompF. E. coli cells harbouring an expression vector containing these genes downstream of a tac promoter were able to secrete a Fab fragment of the antibody efficiently. When the cells were cultured at 37 degrees C and the inducer (isopropyl-beta-D-thiogalactopyranoside, IPTG) concentration was 1 mM (standard conditions), production of functional Fab was very low (medium; 200 ng/l culture and periplasm; less than 90 ng/l culture). In order to optimize functional Fab production, we examined the influence of culture conditions (i.e. temperature and the inducer concentration) on secretion of the product. It was found that a 12.7-fold higher amount of Fab fragment could be produced at 30 degrees C using 0.1 mM IPTG, as compared with standard conditions. Under these optimal conditions, functional Fab accumulated in the periplasm and culture medium for 10 h after induction and the total production level was found to reach approximately 4.5 mg/l culture.

PMID: 1368908 [PubMed - indexed for MEDLINE]

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1: Protein Eng. 1991 Dec;4(8):971-9. Related Articles, Links

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Related Resources

Secretion and in vivo folding of the Fab fragment of the antibody McPC603 in Escherichia coli: influence of disulphides and cis-prolines.

Skerra A, Pluckthun A.

Genzentrum der Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, Germany.

Using the well-characterized antibody McPC603 as a model, we had found that the Fv fragment can be isolated from Escherichia coli as a functional protein in good yields, whereas the amount of the correctly folded Fab fragment of the same antibody produced under identical conditions is significantly lower. In this paper, we analyse the reasons for this difference. We found that a variety of signal sequences function in the secretion of the isolated chains of the Fab fragment or in the co-secretion of both chains in E.coli. The low yield of functional Fab fragment is not caused by inefficient expression or secretion in E.coli, but by inefficient folding and/or assembly in the periplasm. We compared the folding yields for the Fv and the Fab fragment in the periplasm under various conditions. Several diagnostic framework variants were constructed and their folding yields measured. The results show that substitutions affecting cis-proline residues and those affecting various disulphide bonds in the protein are by themselves insufficient to dramatically change the partitioning of the folding pathway to the native structure, and the cause must lie in a facile aggregation of folding intermediates common to all structural variants. However, all structural variants could be obtained in native form, demonstrating the general utility of the secretory expression strategy.

PMID: 1817261 [PubMed - indexed for MEDLINE]

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1: Gene. 1984 Mar;27(3):315-22. Related Articles, Links

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Secretion and processing of an immunoglobulin light chain in Escherichia coli.

Zemel-Dreasen O, Zamir A.

PubMed Services

When a cDNA coding for the kappa light chain (L-321) from the mouse MOPC321 myeloma was cloned into Escherichia coli, L-321 antigens were found in both cytoplasmic and periplasmic fractions. In cells synthesizing the intact chain, starting with its signal peptide, the periplasm contained a mature-size immunoglobulin indicating that the eukaryotic signal peptide can initiate secretion and be processed. When the entire cDNA for L-321 (including its signal peptide) was inserted in the gene for bacterial beta-lactamase, processing cleaved only the first bacterial signal sequence of the hybrid protein synthesized. Removal of the beta-lactamase signal peptide was also observed with another beta-lactamase-L-321 hybrid which did not include the immunoglobulin signal peptide and the adjacent part of the variable region. The two hybrid proteins may, however, differ in their mode of secretion.

Related Resources

PMID: 6428978 [PubMed - indexed for MEDLINE]

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1: J Cell Sci Suppl. 1989;11:45-57. Related Articles, Links

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A novel C-terminal signal sequence targets Escherichia coli haemolysin directly to the medium.

Gray L, Baker K, Kenny B, Mackman N, Haigh R, Holland IB.

Department of Genetics, University of Leicester, UK.

Escherichia coli haemolysin (HlyA), a 107K (K = 10(3) Mr) protein, is secreted to the medium in an hlyB, hlyD-dependent process. Secretion, however, depends on neither an N-terminal signal sequence nor on SecA, which is part of the normal cellular export machinery for periplasmic and outer membrane proteins. In contrast, HlyA contains a novel C-terminal secretion signal encompassing the last 27 amino acids and possibly some additional residues immediately upstream. This region is characterized by a 16 residue 'aspartic acid box' composed largely of small amino acids which we propose constitutes an important element in recognition of the membrane translocation complex constituted by HlyB and HlyD. This feature is also found at the C-terminus of the adenyl cyclase and leukotoxin A molecules and resembles a recently identified eukaryotic C-terminal signal for targeting to glycosomes. A domain of the HlyB component of the haemolysin transport system is also similar to a domain widely distributed in nature, apparently acting as an ATP-dependent transport protein for a wide variety of molecules. Secretion of haemolysin, however, is the first example of a protein translocation system involving an HlyB-like molecule. This suggests that a major role of HlyB or at least its C-terminal domain is the coupling of energy to translocation of the haemolysin. It is more likely therefore that HlyD is more involved in the actual translocation through the membrane. On the basis of genetical and biochemical studies we propose that the haemolysin is translocated directly to the medium bypassing the periplasm. We further propose that HlyB and HlyD together constitute a membrane-bound translocator specific for molecules bearing the HlyA targeting sequence, and that the organization of this complex (conceivably involving other E. coli membrane proteins) must somehow straddle the inner and outer membranes. Finally, the HlyA C-terminal domain has been successfully used to promote the secretion to the medium of a number of heterologous polypeptides, in an HlyB,D-dependent manner.

Publication Types:
- Review
- Review, Tutorial

1: Protein Sci. 1997 Oct;6(10):2180-7. Related Articles, Links

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Glutathione S-transferase can be used as a C-terminal, enzymatically active dimerization module for a recombinant protease inhibitor, and functionally secreted into the periplasm of Escherichia coli.

Tudyka T, Skerra A.

Institut fur Biochemie, Technische Hochschule, Darmstadt, Germany.

Glutathione S-transferase (GST) from Schistosoma japonicum, which is widely used for the production of fusion proteins in the cytoplasm of Escherichia coli, was employed as a functional fusion module that effects dimer formation of a recombinant protein and confers enzymatic reporter activity at the same time. For this purpose GST was linked via a flexible spacer to the C-terminus of the thiol-protease inhibitor cystatin, whose binding properties for papain were to be studied. The fusion protein was secreted into the bacterial periplasm by means of the OmpA signal peptide to ensure formation of the two disulfide bonds in cystatin. The formation of wrong crosslinks in the oxidizing milieu was prevented by replacing three of the four exposed cysteine residues in GST. Using the tetracycline promoter for tightly controlled gene expression the soluble fusion protein could be isolated from the periplasmic protein fraction. Purification to homogeneity was achieved in one step by means of an affinity column with glutathione agarose. Alternatively, the protein was isolated via streptavidin affinity chromatography after the Strep-tag had been appended to its C terminus. The GST moiety of the fusion protein was enzymatically active and the kinetic parameters were determined using glutathione and 1-chloro-2,4-dinitrobenzene as substrates. Furthermore, strong binding activity for papain was detected in an ELISA. The signal with the cystatin-GST fusion protein was much higher than with cystatin itself, demonstrating an avidity effect due to the dimer formation of GST. The quaternary structure was further confirmed by chemical crosslinking, which resulted in a specific reaction product with twice the molecular size. Thus, engineered GST is suitable as a moderately sized, secretion-competent fusion partner that can confer bivalency to a protein of interest and promote detection of binding interactions even in cases of low affinity.

PMID: 9336840 [PubMed - indexed for MEDLINE]

1: Trends Biotechnol. 1994 Nov;12(11):450-5. Related Articles, Links

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PubMed Services

Related Resources

Heterologous protein secretion and the versatile Escherichia coli haemolysin translocator.

Blight MA, Holland IB.

Institut de Genetique et Microbiologie, Universite de Paris-Sud, Orsay, France.

Heterologous proteins synthesized in the Gram-negative bacterium Escherichia coli in bioreactor culture may accumulate in one of three 'compartments':the cytoplasm, the periplasm, or the extracellular medium. Many overexpressed proteins from various origins have been purified from each of these locations. However, to date, each system has required specific tailoring to meet the stringent requirements for each protein product to ensure correct folding, activity and appropriate yield. The E. coli haemolysin secretion system appears to provide a flexible mechanism with which to secrete a wide variety of heterologous fusion proteins into the extracellular medium.

Publication Types:
- Review
- Review, Tutorial

PMID: 7765544 [PubMed - indexed for MEDLINE]

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1: J Chromatogr A. 1994 Aug 5;676(2):337-45. Related Articles, Links

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One-step affinity purification of bacterially produced proteins by means of the "Strep tag" and immobilized recombinant core streptavidin.

Schmidt TG, Skerra A.

Abteilung Molekulare Membranbiologie, Max-Planck-Institut fur Biophysik, Frankfurt am Main, Germany.

The "Strep tag" is a nine amino acid peptide with intrinsic streptavidin-binding activity. If this sequence is genetically fused to the C-terminus of a polypeptide the recombinant protein can be directly purified by affinity chromatography from the host cell extract on immobilized streptavidin. However, variations were observed in the suitability of different commercial streptavidin-agarose preparations for this purpose. Therefore, the influence of the source of streptavidin, the coupling chemistry, and the nature of the affinity chromatography resin was investigated. A procedure was developed for the production of recombinant core streptavidin in Escherichia coli, followed by its efficient refolding and purification with an overall yield of up to 140 mg functional protein per 11 bacterial culture. When coupled to activated CH-Sepharose 4B this truncated form of streptavidin showed a performance in the affinity chromatography of Strep tag fusion proteins that was superior to all other combinations tested. In contrast to its conventional preparation from Streptomyces strains the recombinant core streptavidin was produced without a proteolytic processing step. Thus, deleterious effects during the streptavidin affinity purification of proteins due to residual proteolytic activity in the immobilized streptavidin were avoided. The versatility of the optimized purification system was demonstrated with five different Strep tag fusion proteins.

PMID: 7921186 [PubMed - indexed for MEDLINE]

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1: Protein Expr Purif. 1994 Oct;5(5):509-17. Related Articles, Links

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In vivo biotinylated recombinant antibodies: construction, characterization, and application of a bifunctional Fab-BCCP fusion protein produced in Escherichia coli.

Weiss E, Chatellier J, Orfanoudakis G.

Ecole Superieure de Biotechnologie de Strasbourg, Illkirch, France.

We describe a novel vector system suitable for the efficient preparation of in vivo biotinylated antibody Fab fragments in Escherichia coli. The previously described pGE20 vector used for the functional expression of truncated heavy (Fd) and light (L) chains of Fab into the bacterial culture medium was modified by inserting the C-terminal 101-amino-acid polypeptide of the biotin carboxyl carrier protein subunit of E. coli acetyl-CoA carboxylase (BCCP*). The secreted Fd-BCCP* fusion and L chain proteins were found to be disulfide linked and Fab-BCCP* complexes of an IgG1 antibody (Mab4) to human tumor necrosis factor alpha (TNF) were shown to retain both antigen and streptavidin-binding activities. The capacity of the Fab4 linked to BCCP* to bind TNF was identical to that observed with unmodified Fab4. Up to 15% of the expressed hybrids were able to interact with streptavidin when exogeneous d-biotin was added into the bacterial culture medium. The Fab4-BCCP* molecules were found to be more efficient than Fab4 linked to an engineered streptavidin-affinity tag for the detection of antigen on solid phase. In addition, we show here that the bacterially expressed Fab4-BCCP* complexes, adsorbed to streptavidin-agarose beads, can be used for the one-step purification of recombinant TNF by immunoaffinity chromatography.

PMID: 7827508 [PubMed - indexed for MEDLINE]

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1: J Mol Biol. 1996 Feb 9;255(5):753-66. Related Articles, Links

Molecular interaction between the Strep-tag affinity peptide and its cognate target, streptavidin.

Schmidt TG, Koepke J, Frank R, Skerra A.

Max-Planck-Institut fur Biophysik, Frankfurt/Main, Germany.

The Strep-tag is a selected nine-amino acid peptide (AWRHPQFGG) that displays intrinsic binding affinity towards streptavidin and has been used as an affinity tag for recombinant proteins. In order to elucidate the molecular mechanism underlying this type of artificial protein-peptide recognition, X-ray crystallographic analyses and binding measurements were carried out. The crystal structure of the complex between recombinant core streptavidin and the synthesized peptide was solved and refined at 1.7 A resolution (space group I4(1)22; unit cell dimensions a = b = 58.3 A, c = 176.9 A). The Strep-tag was bound at the same surface pocket where biotin, the natural ligand of streptavidin, gets complexed. The peptide backbone exhibited 3(10)-helical conformation, with eight of the residues involved in protein contacts. The C-terminal Gly-Gly moiety of the Strep-tag participated in a salt bridge to Arg84 of streptavidin with its free carboxylate group. This finding explained why the use of the Strep-tag in fusions with recombinant proteins was restricted to their carboxyl end. Employing a synthetic peptide spot assay, the variant Strep-tag II was screened, which did not have this limitation. The isomorphous crystal structure of its complex with streptavidin revealed that a glutamate side-chain provided the salt bridge in this case, with an otherwise almost unchanged mode of binding. Affinity constants between the peptides and streptavidin were measured by isothermal titration calorimetry. A value of 2.7 x 10(4) M-1 was determined for the Strep-tag peptide, and slightly tighter binding was seen when the Strep-tag was applied as part of a bacterially produced fusion protein. This affinity is significantly higher, compared with values previously reported for shorter streptavidin-binding peptides, and agrees well with the remarkable selectivity observed in recombinant protein purification applications.

PMID: 8636976 [PubMed - indexed for MEDLINE]

1: Bioconjug Chem. 1998 Nov-Dec;9(6):725-35. Related Articles, Links

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In vitro enzymatic biotinylation of recombinant fab fragments through a peptide acceptor tail.

Saviranta P, Haavisto T, Rappu P, Karp M, Lovgren T.

Department of Biotechnology, University of Turku, Tykistokatu 6, FIN-20520 Turku, Finland. Petri.saviranta@utu.fi

We describe the site-specific enzymatic biotinylation of recombinant anti-estradiol Fab fragments through a 13 amino acid acceptor peptide translationally fused to the C-terminus of the Fd chain. The Fab-peptide fusion proteins were secreted to the periplasm of Escherichia coli, purified, and biotinylated in vitro using biotin ligase, biotin, and ATP. The E. coli biotin ligase (the BirA protein) was produced as a novel N-terminal fusion protein with glutathione S-transferase (GST) and purified in one step from bacterial cell lysate using a Glutathione Sepharose affinity column. The purified fusion protein worked as such (without cleavage of the GST part) for the in vitro biotinylation of the Fab fragments. After the removal of nonbiotinylated Fab fragments by monomeric avidin chromatography, the overall yield of biotinylated Fab was 40%. The site-specifically biotinylated Fab fragments (BioFab) were tested in streptavidin-coated microtitration wells, to which they were shown to bind linearly with respect to the amount of BioFab added, specifically as indicated by biotin inhibition, and tightly with a half-life of several days. Moreover, the enzymatic BioFab exhibited uniform antigen binding affinity unlike the same recombinant Fab fragments biotinylated through random chemical conjugation to surface lysines. Finally, the BioFab demonstrated its potential as a well-behaving immunoassay reagent in a model competitive assay for estradiol.

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Affinity enhancement of a recombinant antibody: formation of complexes with multiple valency by a single-chain Fv fragment-core streptavidin fusion.

Kipriyanov SM, Little M, Kropshofer H, Breitling F, Gotter S, Dubel S.

Recombinant Antibody Research Group, Heidelberg, Germany.

In antigen-antibody interactions, the high avidity of antibodies depends on the affinity and number of the individual binding sites. To develop artificial antibodies with multiple valency, we have fused the single-chain antibody Fv fragments to core streptavidin. The resulting fusion protein, termed scFv::strep, was found after expression in Escherichia coli in periplasmic inclusion bodies. After purification of the recombinant product by immobilized metal affinity chromatography, refolding and size-exclusion FPLC, tetrameric complexes resembling those of mature streptavidin were formed. The purified tetrameric scFv::strep complexes demonstrated both antigen- and biotin-binding activity, were stable over a wide range of pH and did not dissociate at high temperatures (up to 70 degrees C). Surface plasmon resonance measurements in a BIAlite system showed that the pure scFv::strep tetramers bound immobilized antigen very tightly and no dissociation was measurable. The association rate constant for scFv::strep tetramers was higher than those for scFv monomers and dimers. This was also reflected in the apparent constants, which was found to be 35 times higher for pure scFv::strep tetramers than monomeric single-chain antibodies. We could also show that most of biotin binding sites were accessible and not blocked by biotinylated E.coli proteins or free biotin from the medium. These sites should therefore facilitate the construction of bispecific multivalent antibodies by the addition of biotinylated ligands.

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Increased streptavidin uptake in tumors pretargeted with biotinylated antibody using a conjugate of streptavidin-fab fragment.

Yao Z, Zhang M, Sakahara H, Saga T, Kobayashi H, Nakamoto Y, Toyama S, Konishi J.

Department of Nuclear Medicine, Faculty of Medicine, Kyoto University, Japan. yao@kuhp.kyoto-u.ac.jp

Radiolabeled streptavidin accumulated in tumors pretargeted with biotinylated antibody. However, the absolute delivery of radioactivity was limited. To increase the tumor uptake of radioactivity further, we conjugated streptavidin with a mouse monoclonal antibody (MAb) fragment, OST6Fab, which recognizes antigen on human osteosarcoma. Another mouse MAb, OST7, which also reacts with the same tumor but recognizes an epitope different from the OST6 epitope, was biotinylated. The radioiodinated streptavidin-OST6Fab conjugate was administered to tumor-bearing mice after the biotinylated OST7 pretargeting. The uptake of the conjugate in tumors pretargeted with the biotinylated antibody was significantly higher than that of streptavidin and that of the conjugate of streptavidin and irrelevant Fab fragment. Renal uptake of radioactivity was decreased markedly, and the blood clearance was retarded by the conjugation with Fab fragment. In conclusion, the conjugate of streptavidin with specific Fab fragment increased the accumulation of radioactivity in tumors pretargeted with biotinylated antibody.

PMID: 9751423 [PubMed - indexed for MEDLINE]

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1: Gene. 1992 Dec 15;122(2):361-5. Related Articles, Links

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Production and secretion of a bifunctional staphylococcal protein A::antiphytochrome single-chain Fv fusion protein in Escherichia coli.

Gandecha AR, Owen MR, Cockburn B, Whitelam GC.

Department of Botany, University of Leicester, UK.

A bifunctional molecule was genetically engineered which contained the secretory signal and four Fc-binding domains of Staphylococcus aureus protein A (FcA), fused to a single-chain Fv (scFv) derived from an immunoglobulin (Ig) G1 mouse monoclonal antibody (AS32) directed against the plant regulatory photoreceptor protein, phytochrome. The FcA::AS32scFv sequence was encoded in a single synthetic gene and expressed as a 60-kDa periplasmic protein in Escherichia coli. The bifunctionality of the fusion protein was established by its ability to bind to both IgG-agarose and phytochrome-sepharose. Growth of cultures, producing the FcA::AS32scFv, at 37 degrees C, resulted in a decrease in the periplasmic accumulation of the fusion protein, and an increased accumulation of an assumed degradation product which retained Fc-binding activity. Growth of cultures at lower temperatures favoured the accumulation of undegraded fusion protein. The recombinant fusion protein could be purified to homogeneity by a simple, rapid chromatography procedure.

PMID: 1487150 [PubMed - indexed for MEDLINE]

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1: Protein Expr Purif. 1998 Nov;14(2):192-6. Related Articles, Links

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Production of a soluble and functional recombinant streptavidin in Escherichia coli.

Gallizia A, de Lalla C, Nardone E, Santambrogio P, Brandazza A, Sidoli A, Arosio P.

Dibit, Department of Biological and Technological Research, San Raffaele Scientific Institute, Milan, 20132, Italy.

The cDNA for streptavidin (residues 15-159) was subcloned into an expression vector in fusion at the N-terminus with the T7-tag (12 residues). Conditions were found to express the protein in Escherichia coli in a soluble, assembled, and active form. The protein was purified in two simple steps which involved heating at 75 degreesC and affinity chromatography on iminobiotin agarose. The purified protein was obtained in yields of 70 mg per liter of bacterial culture. Electron spray mass spectrometry analysis showed that the recombinant streptavidin had the expected molecular mass without covalent modifications. ELISA and surface plasmon resonance analyses showed it to be functionally analogous to the natural streptavidin. This appears to be an improvement over the reported methods of recombinant streptavidin production which involve protein renaturation or the use of eukaryotic expression systems. Copyright 1998 Academic Press.

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1: Bioorg Khim. 1999 Mar;25(3):184-8. Related Articles, Links

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[Cloning of streptavidin gene from Streptomyces avidinii and its expression in Escherichia coli. Secretion of streptavidin by E. coli cells]

[Article in Russian]

Veiko VP, Gul'ko LB, Okorokova NA, D'iakov NA, Debabov VG.

State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russia. veiko@vnigen.msk.su

The streptavidin gene from Streptomyces avidinii was cloned, an expression plasmid constructed, and a highly effective strain producer of streptavidin created. It was shown that the leader peptide of streptavidin ensures the effective secretion of this protein into the periplasmic space of Escherichia coli cells. The degradation site of the leader peptide was detected. Upon treatment with the total fraction of proteases secreted by S. avidinii into the culture medium, "core" streptavidin was obtained, which retained the biotin-binding function.

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Identification of biotinylated molecules using a baculovirus-expressed luciferase-streptavidin fusion protein.

Karp M, Lindqvist C, Nissinen R, Wahlbeck S, Akerman K, Oker-Blom C.

University of Turku, Finland.

A genetic fusion between streptavidin of Streptomyces avidinii and luciferase of Pyrophorus plagiophthalamus was constructed. The fusion protein was produced in the Sf9 insect cell line using the baculovirus expression vector system (BEVS). Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the proteins from cells infected with the recombinant virus, VL1393-LucGR-StreptAv, revealed that the fusion protein migrated with an apparent molecular weight of 75 kDa. Light emission measurements showed that the infected cells produced about 255 mg of the chimeric protein per liter of cell culture (127.5 micrograms/1 x 10(6) cells). Precipitation of the LucGR-StreptAv fusion protein with biotinylated acrylic beads as well as immunoblot analyses using biotinylated immunoglobulins indicated that both fusion moieties of the chimeric protein product were functional with respect to their physical and enzymatic activities.

PMID: 8679206 [PubMed - indexed for MEDLINE]

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1: Biochem Biophys Res Commun. 1991 Apr 30;176(2):571-7. Related Articles, Links

Expression vectors for streptavidin-containing chimeric proteins.

Sano T, Cantor CR.

Department of Molecular and Cell Biology, University of California, Berkeley.

We have constructed expression vectors for streptavidin-containing chimeric proteins. These vectors carry the DNA sequence corresponding to the core region of the streptavidin molecule, and have several unique cloning sites which facilitate construction of gene fusions of streptavidin with a target protein. A chimeric protein of streptavidin and the target protein should be expressible in Escherichia coli by using the T7 expression system. Because of the strong and specific biotin-binding affinity of the streptavidin moiety, such streptavidin-containing chimeric proteins should extensively expand the applications of the streptavidin-biotin system, and offer a variety of applications as new biological tools.

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Functional and pharmacokinetic properties of antibody-avidin fusion proteins.

Shin SU, Wu D, Ramanathan R, Pardridge WM, Morrison SL.

Institute of Environment and Life Science, The Hallym Academy of Sciences, Hallym University, Kangwon-do, Korea.

In an attempt to produce broadly useful targeting agents, genetic engineering and expression techniques have been used to produce Ab-avidin fusion proteins. Chicken avidin has been fused to mouse-human chimeric IgG3 at the end of C(H)1 (C(H)1-Av), immediately after the hinge (H-Av), and at the end of C(H)3 (C(H)3-Av). Fusion heavy chains of the expected molecular mass were expressed, assembled with a co-expressed light chain, and secreted. The resulting molecules continued to bind Ag. They also bound biotinylated human serum albumin; C(H)3-Av had reduced affinity (K(A) = 5.13 x 10(9) M(-1)) compared with the tetrameric avidin (K(A) = 1 x 10(15) M(-1)), but greater affinity than monomeric avidin (K(A) = 1 x 10(7) M(-1)). Importantly, the avidin-IgG fusion proteins had a longer serum t1/2 in rats than avidin. The favorable pharmacokinetic parameters suggest that these avidin fusion proteins can be used effectively to deliver biotinylated ligands such as drugs and peptides to locales expressing any Ag recognized by the associated Ab.

PMID: 9144494 [PubMed - indexed for MEDLINE]